SYNERGY OR COMBINED EFFECT? A CRITICAL ANALYSIS OF PHYTOGENIC COMBINATIONS USED IN BROILER CHICKEN DIETS UNDER HEAT STRESS

D.-C. Negură*, M. G. Usturoi

"Ion Ionescu de la Brad" Iasi University of Life Sciences, Romania

Abstract

The use of phytogenic additives represents a promising nutritional strategy for mitigating the effects of high-temperature exposure in broiler chickens, given their antioxidant, anti-inflammatory, and immunomodulatory effects, as well as their role in supporting intestinal homeostasis. Combined phytogenic strategies are of particular interest, based on the premise that their complementary mechanisms may produce greater effects than when administered individually. However, demonstrating the beneficial effect of a mixture does not, in itself, prove synergism, raising an important interpretive question: when can the effect of a mixture be considered truly synergistic? This paper critically analyses the evidence regarding the use of such combinations, examining the relationship between the composition of the mixtures, their effects on productive performance and physiological responses, as well as distinguishing between combined effects and potentially synergistic ones. The evidence from the available literature is more consistent regarding the efficacy of the combinations, while the synergistic nature of the combinations remains limited, thus necessitating factorial experiments, direct comparisons between the mixture and its components, and an analysis of interactions to demonstrate synergism, as well as its relevance to the physiological adaptability and production performance of broiler chickens subjected to heat stress.

Key words: *phytogenic additives, poultry, bioactive compounds, complementary effects*

INTRODUCTION

Heat stress is one of the most significant environmental factors that negatively affect the welfare, health, and production performance of broiler chickens, thus posing a major challenge to the sustainability and profitability of the poultry sector worldwide. Exposure of birds to high ambient temperatures triggers a series of metabolic, physiological, and behavioural changes, thereby negatively affecting growth rate, feed intake, feed conversion efficiency, and ultimately, meat quality [1; 2; 3].

The increased susceptibility of broiler chickens to high temperatures is closely linked to genetic progress resulting from intensive genetic selection aimed at achieving accelerated growth rates and high carcass yield. This vulnerability has been associated with increased metabolic activity and, consequently, with increased endogenous heat production; physiological thermoregulatory mechanisms become insufficient to maintain thermal homeostasis, thereby promoting the onset of heat stress and associated metabolic imbalances [1; 4].

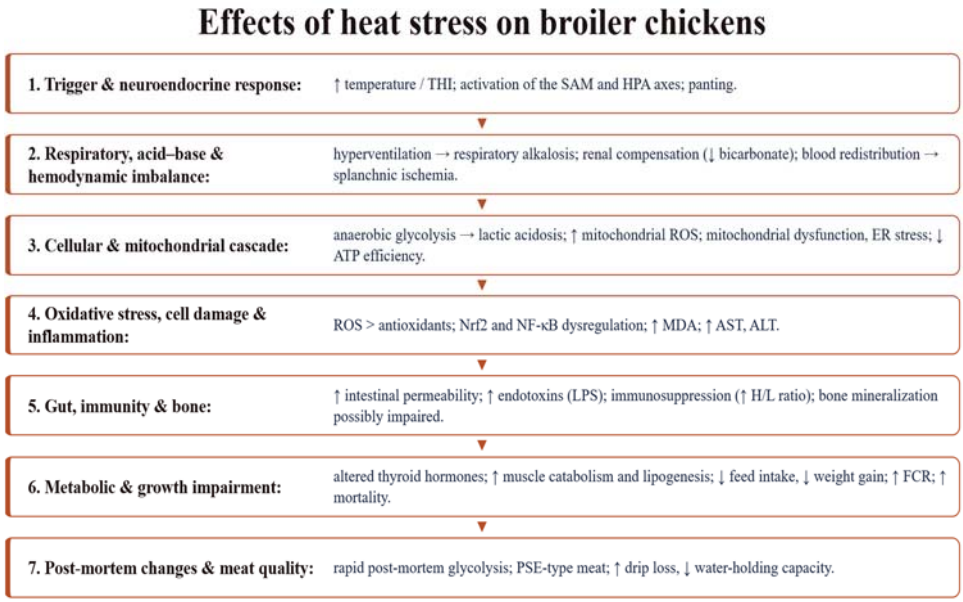
Oke et al. (2024) highlight that climate change, manifested by rising ambient temperatures and more frequent periods of extreme heat, increases broilers' vulnerability to heat, not only in tropical and subtropical areas, but is also becoming

*Corresponding author: delia.negura@iuls.ro
The manuscript was received: 17.10.2026
Accepted for publication: 17.11.2026

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

an increasingly important concern in poultry production systems in temperate regions [5].

Depending on the duration and characteristics of exposure to high temperatures, Nawaz et al. (2021) classify heat stress as acute or chronic [2]. Acute heat stress is associated with short-term exposure to high temperatures, which can trigger rapid physiological responses, while chronic heat stress involves prolonged exposure to unfavorable conditions. Depending on the temporal distribution of exposure, chronic heat stress can be cyclical (when high temperatures alternate with periods of thermal comfort) or constant (when exposure to heat is continuous) [6]. The body's response to heat manifests as a series of interdependent changes (Figure 1).



ATP - adenosine triphosphate; ALT - alanine aminotransferase; AST - aspartate aminotransferase; FCR - feed conversion ratio; H/L - heterophil-to-lymphocyte ratio; HPA - hypothalamic-pituitary-adrenal axis; LPS - lipopolysaccharides; MDA - malondialdehyde; PSE - pale, soft, exudative meat; RE - endoplasmic reticulum; ROS - reactive oxygen species; SAM - sympathoadrenomedullary axis; THI - temperature-humidity index.

Figure 1 The effects of heat stress on broiler chickens, from heat load to meat quality [6; 7; 8; 2; 9; 10]

Due to the high costs associated with implementing and maintaining shelters with controlled microclimates and modern ventilation systems [2], new research is focusing on complementary nutritional strategies, which are considered a promising approach for reducing the effects of heat stress. This situation has spurred intensive research into phytogenic additives, plants, spices, and plant-derived products, as well as plant extracts and essential oils [11] which are rich in phytochemicals such as flavonoids, polyphenols, alkaloids, terpenoids, and organosulfur compounds [6;12;10;13;7]. The potential of bioactive compounds to improve the productive performance of broiler chickens subjected to heat stress is associated with their ability to reduce oxidative stress, protect intestinal integrity, and promote efficient nutrient utilization [6; 14; 15; 17].

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

This paper aims to highlight that a phytogenic mixture can be effective without demonstrating synergy among its components, as the literature typically addresses whether the mixture works without providing an answer regarding the interaction of its components. The goal is to critically analyse in vivo studies on heat-stressed broiler chickens in which phytogenic combinations were tested, assessing whether the experimental design and statistical analysis allow for conclusions regarding synergy or merely describe a combined effect.

MATERIAL AND METHOD

This literature review is designed as a comparative and critical narrative review aimed at evaluating and interpreting the results of in vivo studies on broiler chickens subjected to heat stress. The data were taken from original articles, without recalculating the authors' statistics. Following a literature search in scientific databases and search engines with access to specialized literature (Google Scholar, Scopus, Web of Science, PubMed), 26 studies were identified; after eliminating 3 duplicates, 23 unique articles remained, all of which were analysed in full. Of these, 8 articles were used to provide the theoretical foundation for this study, and 15 studies met the eligibility criteria and were included in this comparative analysis. The selection of studies was based on the following inclusion criteria: in vivo experiments on broiler chickens (studies on laying hens were not included); documented heat stress, with a control group subjected to the same heat stress; testing of a phytogenic mixture; and original data. The following information was extracted from each study: the composition of the mixture and the type of combination; the heat stress model (type of exposure, number of hours per day, temperature, age at onset, duration of exposure, relative humidity, presence of a thermoneutral group); characteristics of the birds and the experimental design (hybrid, sex, number, number of groups and replicates, duration of the experiment); experimental groups (heat-stressed control, individual components/mixtures); parameters (mortality, productive performance, carcass, meat quality, serum biochemistry, haematology, immunity, oxidative stress, gut and microbiota, stress indicators, heat shock proteins, hormones) and testing of interactions between components. The combinations were grouped into the following categories: plant + plant; essential oils/purified compounds; plant + non-plant component (probiotic, vitamin C, flaxseed oil).

DISCUSSIONS

Given the diversity of bioactive compounds with antioxidant, anti-inflammatory, and immunomodulatory properties, as well as the physiological mechanisms that can influence the body's response to high temperatures, the use of phytogenic additives in nutritional strategies aimed at reducing the effects of heat stress [17]. However, experimental results are not always consistent, and the observed effects can vary considerably depending on the phytochemical composition, the administered dose, the nature of the additive, and the specifics of the experiment conducted [18]. Akbarian et al. (2017) highlighted that certain flavonoids can exert beneficial effects under heat stress, but under thermoneutral conditions, their benefits may be weaker or even neutral [6]. Even among additives derived from the same botanical species, the proportion of bioactive compounds may vary depending on growing conditions, plant material, extraction method, and the part of the plant used [6].

In the reviewed literature, the same combination was not tested in multiple studies; each mixture was evaluated only once using different plants, doses, formulations, and heat stress models. Ghazi

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

et al., 2015 combined oregano oil (57.7% carvacrol) with vitamin C [19], Elbaz et al., 2013 combined clove oil (69.7% eugenol) with probiotics [20], and Kamboh et al., 2013 administered genistein and hesperidin in purified form [21]. Under these conditions, the results cannot be grouped by a specific compound or plant, which makes it impossible to determine the individual role of the compounds that exert a specific effect on heat-stressed broilers.

For combinations, the reported results vary from one study to another, confirming that they cannot be interpreted in a generalized manner. The mixture of blueberry leaves, mint, fennel, and sea buckthorn meal administered by Saracila et al. (2020) did not alter productive performance but increased hepatic antioxidant activity [22]. Attia et al., 2023, reported that the combination of *Spirulina platensis* and garlic improved intestinal morphology and microbial balance [23], while Elbaz et al., 2023, highlighted that the administration of clove essential oil combined with probiotics improves the feed conversion ratio [20]. A different perspective on herbal combinations included in broiler diets is provided by the research conducted by Abou-Kassem et al., (2025), in which the combination of green tea and ginger did not enhance the effect of the individual components, and higher doses actually reduced growth compared to separate administration [24]. The beneficial effect observed compared to the control group does not, in and of itself, indicate whether the combined action of the components exceeds the sum of their separate actions. This concept originates in pharmacology, based on the hypothesis that the use of combinations aims to achieve greater efficacy and reduced toxicity [25], and the analytical methods developed [26; 27] can be applied to animal nutrition. Thus, combining multiple plants or bioactive compounds has become a common practice in poultry nutrition, based on the premise that their combined action can enhance the effects of each component [22], and understanding the interactions between additives is essential for formulating effective mixtures [28].

Heat stress models and experimental designs. In the studies analyzed, the experimental models for inducing heat stress showed considerable variability, due to differences in the birds age at the onset of heat stress, as well as the duration, frequency, and intensity of heat stress exposure. The experimental conditions were recorded based on data provided by the authors of the original studies, with several types of exposure identified: continuous, cyclic, chronic with daily fluctuations, ambient, and under natural summer conditions, characterized by the temperature - humidity index (THI). This classification was developed solely to compare experimental characteristics and organize them, given that certain heat stress models may share characteristics common to multiple categories. The term “chronic” describes prolonged exposure, while “cyclic” refers to the alternation of low and high temperatures.

The evaluated parameters were grouped into 10 domains (Figure 2); production performance was reported in 13 studies, serum biochemistry and carcass traits in 9 studies, and oxidative stress in 10 studies. Hematology, gut/microbiota, and stress indicators (body temperature, hormones, heat shock proteins) were evaluated in 5 studies; mortality/survival and immune indicators in 5 studies; and meat quality in 3 studies. Most of these areas were covered by Attia et al., 2023, except for meat quality [23]. In contrast, the study by Pirmohamammadi et al., 2016, assessed only meat quality [29], and Fayed et al., 2024, did not report on productive performance [30].

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

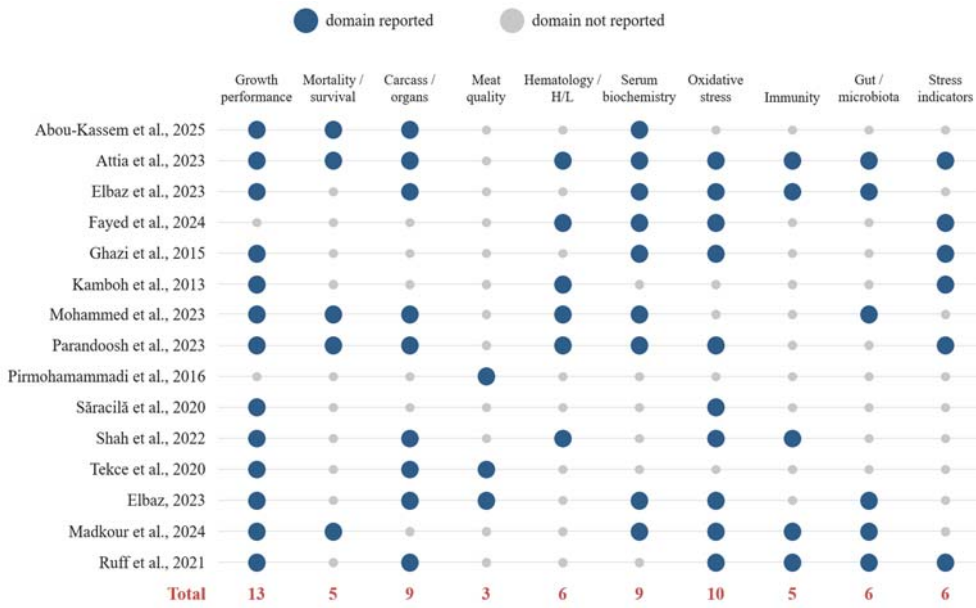


Figure 2 Main outcomes assessed in the included studies

Continuous exposure models involved maintaining high temperatures throughout the experimental period, without intervals of return to thermoneutral conditions; Mohamed et al. (2023) used a constant temperature of 34°C [31], while Tekce et al. (2020) investigated the effects of exposing broilers to temperatures of 36°C compared to a group maintained at 22°C [32]. The cyclic patterns identified in 7 studies [19; 20; 23; 29; 33; 34] involved daily intervals of exposure to high temperatures followed by periods of lower temperatures; these protocols allowed for the investigation of physiological responses to repeated thermal stress, as well as adaptation and recovery processes between exposure periods.

Kambooh et al., 2013 and Parandoosh et al., 2023 evaluated the effects of heat stress under natural conditions during the hot season, characterized by variations in ambient temperature [21; 35]; Shah et al., 2022 investigated exposure to variable temperatures in a shelter [37], while Madkour et al., 2024, characterized temperature conditions using the THI index [37].

The protocols involved variable temperatures ranging from 32 - 38°C (Table 1), and the duration of cyclic exposure ranged from 4 to 12 hours. Elbaz (2023) and Elbaz et al. (2023) used a regimen of 35°C for 4 hours/day, 5 days/week [20; 34], while Ghazi et al., 2015 applied a temperature of 38°C for 5 hours/day, with additional 4 - hour cooling intervals and 3-hour heating intervals [19]. In the study by Fayed et al., 2024, a temperature of 32°C was maintained for 10 hours between 8:00 a.m. and 6:00 p.m., reduced to 24°C at night [30], while Ruff et al., 2024, used a temperature of 35°C for 12 hours daily between days 7 and 42 [33].

Regarding the age at which broilers are first exposed to high temperatures, this varies from the first day [38; 21] to day 25 [29] and the duration of exposure from day 14 [23] to 45 days [38]. By multiplying the number of hours per day by the number of days of exposure, the cumulative exposure to the stress temperature was estimated in Elbaz, 2023, and Elbaz et al., 2023, to be approximately 94 hours [34; 20], 432 hours in the models with the shortest exposure in

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

Ruff et al., 2021 [33], and 840 - 1,080 hours in the constant temperature models, highlighting a difference of more than tenfold, which limits the comparability of the results.

Relative humidity was reported in ten of the 15 studies, ranging from 45 - 89.5%, and in Elbaz et al., 2023, only for the first 48 hours [20]. The THI was reported only in Madkour et al., 2024, with a mean of 29.19, which the authors classified as severe heat stress (values ranging from 27.37 to 31.08 throughout the experiment) [37]. The thermoneutral group was included in 5 studies [23; 30; 22; 36; 32]. In Săracilă et al., 2020, the thermoneutral group originated from an experiment separate from the heat-stressed one [22], and in Fayed et al., 2024, the group was reared in a separate chamber [30]. In some studies, the physiological response of birds to heat stress was determined using specific stress indicators such as the heterophil - to - lymphocyte (H/L) ratio and plasma corticosterone concentration [23; 21; 35], the expression of the heat shock protein HSP70 [21; 30], behaviour [30], or core body temperature, measured using sensors inserted into the crop.

The commercial hybrids used in the experiments were Ross 308 (8 studies), Cobb 500 (3 studies), Arbor Acres, Hubbard, and Avian - 480 (1 study each). The total number of birds ranged from 60 broilers per experiment [22] to 700 broilers per experiment [36] with 2–9 treatments, 4 - 6 replicates per treatment, and 10–30 birds per replicate (Table 1). The duration of the experiment varied: 35 days (six studies), 42 days in eight studies, and 45 days in the study conducted by Mahommed et al., 2023 [31].

Table 1 Summary of the experimental characteristics of the included studies

Study	Combination	TS	HS	TG	Hybrid (sex) (n)	Group x replicates x birds	SA
[24]	green tea + ginger	Yes	30.3 – 40.7°C	No	Cobb 500, mixed sex, 450	9 x 5 x 10	ANOVA 3x3 factorial with interaction term
[23]	Spirulina + garlic	Yes	35 ± 1°C, 9h/day days 22 – 35	Yes (26°C)	Ross 308, unsexed 600	5 x 6 x 20	ANOVA unifactorial , Tukey
[34]	Fermented pomegranate peel + flaxseed oil	Yes	35°C, 4h/day from 10 day	No	Ross 308, Unsexed 300	4 x 5 x 15	ANOVA unifactorial (GLM)
[20]	Clove EO + probiotic	Yes	35°C, 4h/day from day 10	No	Ross 308, Unsexed 300	4 x 5 x 15	GLM, Tukey
[30]	*Terminalia bellirica* + *Andrographis paniculata (commercial herbal product)*	No	32°C, 10h/day, from week 3	Yes (24°C)	Avian-480 208	4 x 4 x 13	ANOVA unifactorial ,
[19]	EO oregano + vit. C	Yes	38°C, 5h/day from day 21	No	Ross 308, Male 240	4 x 6 x 10	GLM, Duncan

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

Study	Combination	TS	HS	TG	Hybrid (sex) (n)	Group x replicates x birds	SA
[21]	Genistein + hesperidin (1:4)	Yes	34.0 – 37.3°C (day) 24.4 – 27.8°C (night)	No	Arbor Acres. mixt 360	6 x 6 x 10	ANOVA, Tukey-Kramer
[37]	Rosemary extract + oregano	Yes	31.9°C, 35 days UR 50%	No	n.r., male 350	7 x 5 x 10	???
[38]	Black cumin seeds + fenugreek + sesame seeds	No	34°C, days 1-45	No	Ross 308, Male, 330	5 x 6 x 11	GLM, Tukey
[35]	*Ferula gummosa* + *Thymus vulgaris* + *Trachyspermum copticum* (aqueous extract)	No	35,3 – 35,7°C, from day 10	No	Ross 308, Unsexed 400	8 x 5 x 10	ANOVA, factorial
[29]	*Thymus vulgaris* + *Mentha pulegium*	Yes	25 – 42; UR 45%	No	Ross 308, Unsexed 200	4 x 5 x 10	GLM
[33]	*Rozmarinus officinalis* (EO)+ red beet + natural betaine	No	35°C, 12 h/day	No	Cobb 500, male 500	4 x 5 x 25	????
[22]	Blueberry leaves + mint leaves + fennel leaves+ sea buckthorn meal	No	32± 1°C	Yes (≠ trial)	Cobb 500, n.r. 60	2 groups x 30 birds	ANOVA
[36]	Ginger + onion	No	30.5 - 38.8°C, RH 73-90%, from day 8	Yes (24± 3°C)	Hubbard, n.r. 700	5 x 5 x 30	ANOVA unifactorial Tukey
[32]	Eucalyptus + thyme + citronella + cloves (EO blend)	No	36°C, from day 7	Yes (22°C)	Ross 308, male 400	8 x 50	GLM

EO - essential oil; GLM - general linear model; HS - heat stres; n.r - not reported; SA - statistical analysis; TG - thermoneutral group; TS - tested separately; RH – relative humidity.

Combined effect, additivity, synergy, antagonism. Conceptually, these terms must be rigorously distinguished. The combined effect describes the observed response to a mixture without specifying the nature of the interaction; additive, synergistic, and antagonistic effects involve comparing the observed response with the expected response based on the individual components [25; 27]. In the Chou-Talalay method, a combination index equal to 1 defines additivity; a value below 1 indicates

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

synergy; and a value above 1 indicates antagonism (Figure 3) [27]. Several strategies for determining whether a combination is superior to its components have been described in the literature (additivity of the response, comparison with the best single agent, Bliss independence) [26], thus showing that the statistical significance of a combination effect does not, in and of itself, imply a convincing difference from the individual components. The independence model was formulated by Bliss in 1939, initially for the toxicity of substances applied together. In a factorial analysis of variance, the interaction can be tested directly via the interaction term [25].

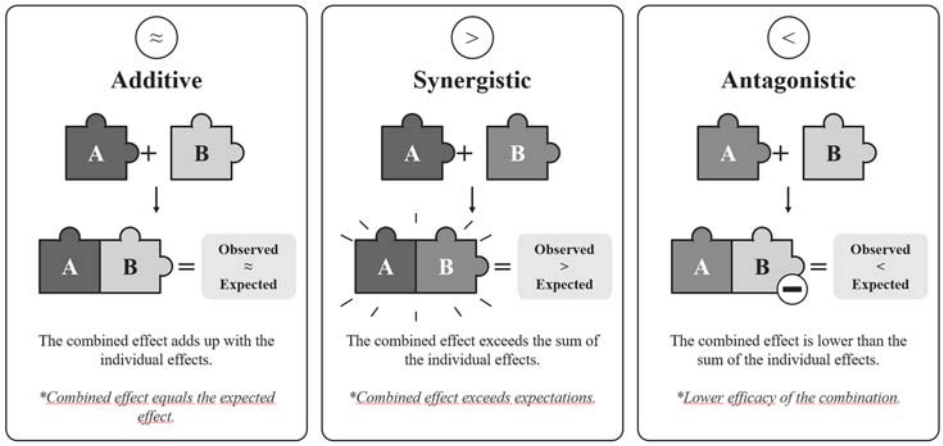


Figure 3 Types of combined effects

The included studies were analysed based on three minimum methodological criteria (Figure 4) to determine the extent to which the experimental design allows for the evaluation of interactions between components:

- the presence of a control group subjected to the same thermal stress;
- testing each component separately;
- direct comparison of the mixture with each component.

The following requirements, derived from the methodological literature [25; 27] were used as a reference framework:

- a control group subjected to thermal stress and, for stress-specific effects, the inclusion of a thermoneutral reference group;
- individual components tested at doses comparable to those in the mixture;
- factorial analysis that includes the interaction term between components;
- direct comparisons between each component and the mixture;
- control of confounding factors, particularly dose differences.

In studies with comparable numerical data, the mixture improved performance compared to the heat-stressed control (Table 2). Thus, Mohammed et al., 2023 reported an 8.1% increase in body weight [31], Elbaz et al., 2023, an 8.7% increase [20], Ghazi et al., 2015, a 9.7% increase [19], Elbaz, 2023, a 9.9% increase [34], and Attia et al., 2023, by 13.5% [23].

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

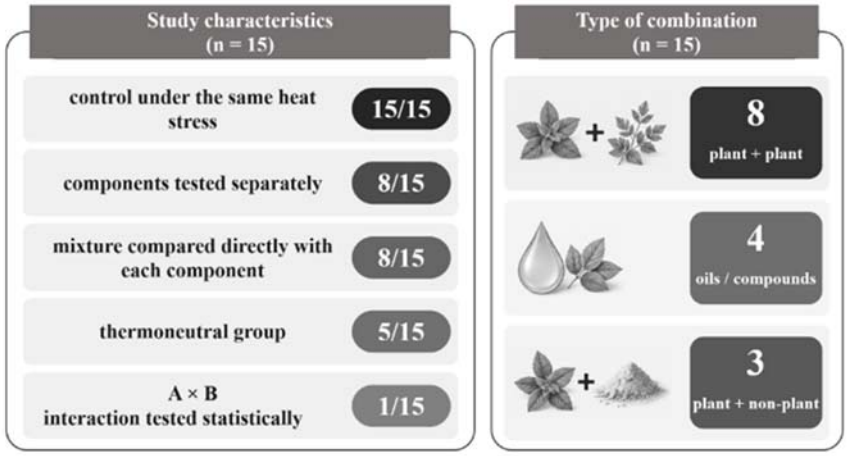


Figure 4 Design characteristics of the included studies:
(left) the number of studies that meet each condition; (right) the distribution by type of combination.
(A.I. generated image)

Table 2 Efficiency of the mixture compared to its components

Study	Combination	Mixture vs. components	Comparative result
[25]	Green tea + ginger	Improved fat and cholesterol levels; FCR unchanged; weight at 4 weeks: 1,446 g vs. 1,521 g.	Mixed results, with no upward trend.
[23]	Spirulina + garlic	BW 2100 g vs. 2035 g and 2020 g; survival rate of 100% vs. 98.3%.	Superior blend.
[34]	Fermented pomegranate peel + flaxseed oil	BW 1957 vs. 1869 g and 1885 g; FCR is superior to both components.	Superior blend.
[20]	Clove EO + Probiotic	BWG 1794 g vs. 1780 g at the best component.	Higher at FCR, comparable in terms of growth.
[19]	Oregano EO + Vitamin C	BW 2,377 g vs. 2,214 g and 2,250 g; daily weight gain and FCR superior to both.	Superior blend.
[37]	rosemary + oregano	The most effective response was to 50 mg/kg of oregano alone;	No superiority.
[21]	genistein + hesperidin (1:4)	BW - hesperidin: 2026 g vs. 2019 g in the mixture (not significant).	No superiority.
[29]	*Thymus vulgaris* + *Mentha pulegium*	pH of the thigh: 6.74 in the mixture, compared to 6.71 and 6.59 (not significant); MDA equal.	No difference (meat quality).

BW – body weight; BWG – body weight gain; FCR – food conversion ratio; MDA – malondialdehyde.

The feed conversion ratio was reduced by 6.4% [20] 7.8% [34], 10.0% [23] and 11.2% [19]. Attia et al., 2023 reported a 100% survival rate in the group administered the mixture [23], while Mahommed et al., 2023 reported a decrease in mortality from 28.8% to 12.1% [31]. Mixed results were also observed: Saracila et al., 2020 reported that a mixture of blueberry leaves, mint, fennel, and sea buckthorn meal did not improve productive performance but increased antioxidant

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

activity in the liver [22], while Madkour et al., 2024 recorded the best response with a single extract [37]

An analysis of the methodological criteria required to assess synergism reveals a significant decrease in the number of studies that meet these conditions. Although all 15 studies used a control group subjected to the same thermal stress, only 8 evaluated the components of the combinations individually. Of these, statistical testing of the interaction between components was performed in only one study [24], which did not reveal any additional effect of the administered combination. Consequently, statistical confirmation of synergy was not achieved in any of the analysed studies. Elbaz et al. (2023), Ghazi et al. (2015), and Kamboh et al. (2013) characterized the effects of the combinations as "synergistic," although the statistical analysis was based exclusively on ANOVA and multiple comparisons between experimental groups, without explicitly testing for interaction between components [20; 19; 21].

Of the studies included in this review, only 7 tested the mixture alone, without testing the individual components [22; 30; 31; 32; 33; 35; 36]. Among the studies that tested the components separately, the dose was not equivalent in Madkour et al., 2024 (double the total dose in the mixture) [37], Kambou et al., 2013 (1 mg/kg genistein in the mixture versus 5 mg/kg alone) [21], and Pirmohamammadi et al., 2016 (0.5% of the two plants combined) [29]. Ruff et al., 2021 tested the essential oil of *Lippia origanoides* separately at 37 ppm versus 45 ppm in a mixture, and rosemary was not tested separately [33]. Three combinations included a non-plant component with its own activity, and two studies, [39; 32], stem from the same experiment.

CONCLUSIONS

The results show that most of the tested mixtures have a beneficial effect on heat-stressed broiler chickens, improving their performance compared to the stressed control group. The significant difference compared to the control group demonstrates the mixture's effectiveness but does not prove its superiority over its individual components [25]. To confirm synergy, the observed response must be compared with the expected response based on the individual components [25; 27], a comparison that is missing or incomplete in 14 of the 15 studies analysed. The response depends on the parameter analysed, and Abou-Kassem et al., 2025, demonstrate that the same mixture can induce favourable changes (lipid parameters and cholesterol levels) without affecting growth [24], while Saracila and colleagues, 2020, reported an increase in hepatic antioxidant activity without altering productive performance [22].

The results cannot be directly compared, partly due to differences in the heat stress models, which vary in temperature (32 - 38°C), whether the stress is constant or cyclic, and duration of exposure (ranging from 94 to 1,080 hours). The duration and intensity of exposure determine the degree of damage [2; 6], leading to different responses in different models for the same administered mixture. Furthermore, the absence of a thermoneutral group (only 5 out of 15 experiments included a thermoneutral group) makes it impossible to distinguish the specific protection against heat stress from the general benefits of the additive.

The importance of this distinction is supported by the observations of Akbarian et al., 2016, according to which the beneficial effects of certain flavonoids did not demonstrate favourable effects on broilers maintained under thermoneutral conditions; their efficacy was evident only under heat stress conditions [6]. Another methodological limitation is the characterization of thermal exposure through the integrated assessment of temperature and humidity (Temperature-

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

Humidity Index, THI), used to facilitate comparisons between experimental conditions. This index was employed in only one study conducted by Madkour et al., 2024, thereby complicating the comparative assessment of exposure severity and the interpretation of differences in the observed physiological and productive responses [37].

Therefore, it can be concluded that a phytogenic mixture may be useful in practice without being synergistic, due to its overall effectiveness; however, the term "synergistic" has a precise mathematical meaning and requires statistical demonstration [25; 27]. A study capable of demonstrating synergy should meet five requirements: (1) testing the components and the combination under the same thermal stress in a factorial design, with analysis of the interaction term [25]; (2) characterization of the individual and combined effects by evaluating multiple dose levels [27]; (3) an appropriate reference model such as Bliss's Independence or the Chou-Talalay method, with statistical validation [26; 27]; (4) the presence of a thermoneutral group to separate stress protection from the general effect of the treatment [6]; (5) monitoring of multiple physiological responses (oxidative stress, immunity, intestinal integrity, growth) [23].

REFERENCES

1. Lara, LJ; Rostagno, MH Impact of heat stress on poultry production. *Animals* **2013**, 3, 356–369. https://doi.org/10.3390/ani3020356
2. Nawaz, AH; Amoah, K; Leng, QY; Zheng, JH; Zhang, WL; Zhang, L Poultry response to heat stress: Its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. *Front. Vet. Sci.* **2021**, 8, 699081. https://doi.org/10.3389/fvets.2021.699081
3. Oluwagbenga, EM; Fraley, GS Heat stress and poultry production: A comprehensive review. *Poult. Sci.* **2023**, 102, 103141. https://doi.org/10.1016/j.psj.2023.103141
4. Apalowo, OO; Ekunseitan, DA; Fasina, YO Impact of heat stress on broiler chicken production. *Poultry* **2024**, 3, 107–128. https://doi.org/10.3390/poultry3020010
5. Oke, OE; Akosile, OA; Uyanga, VA; Oke, FO; Oni, AI; Tona, K; Onagbesan, OM Climate change and broiler production. *Vet. Med. Sci.* **2024**, 10, e1416. https://doi.org/10.1002/vms3.1416
6. Akbarian, A; Michiels, J; Degroote, J; Majdeddin, M; Golian, A; De Smet, S Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *J. Anim. Sci. Biotechnol.* **2016**, 7, 37. https://doi.org/10.1186/s40104-016-0097-5
7. Aryal, B; Kwakye, J; Ariyo, OW; Ghareeb, AFA; Milfort, MC; Fuller, AL; Khatiwada, S; Rekaya, R; Aggrey, SE Major oxidative and antioxidant mechanisms during heat stress-induced oxidative stress in chickens. *Antioxidants* **2025**, 14, 471. https://doi.org/10.3390/antiox14040471
8. Mishra, B; Jha, R Oxidative stress in the poultry gut: Potential challenges and interventions. *Front. Vet. Sci.* **2019**, 6, 60. https://doi.org/10.3389/fvets.2019.00060
9. Olayiwola, SF; Adedokun, SA Heat stress in poultry: The role of nutritional supplements in alleviating heat stress and enhancing gut health in poultry. *Front. Vet. Sci.* **2025**, 12, 1691532. https://doi.org/10.3389/fvets.2025.1691532
10. Wasti, S; Sah, N; Mishra, B Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals* **2020**, 10, 1266. https://doi.org/10.3390/ani10081266
11. Windisch, W; Schedle, K; Plitzner, C; Kroismayr, A Use of phytogenic products as feed additives for swine and poultry. *J. Anim. Sci.* **2008**, 86 (Suppl. 14), E140–E148. https://doi.org/10.2527/jas.2007-0459
12. Goel A., Heat stress management in poultry. *Journal of animal physiology and animal nutrition*, **2021**, 105(6), 1136–1145. https://doi.org/10.1111/jpn.13496
13. Insawake, K; Songserm, T; Songserm, O; Theapparat, Y; Adeyemi, KD; Rassmidatta, K; Ruangpanit, Y. Flavonoids, isoquinoline alkaloids, and their combinations affect growth performance, inflammatory status, and gut microbiome of broilers under high stocking density and heat stress. *Animals (Basel).* **2024**, 15(1):71. doi: 10.3390/ani15010071.

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

14. Surai, PF; Kochish, II; Fisinin, VI; Kidd, MT. Antioxidant defence systems and oxidative stress in poultry biology: an update. *Antioxidants*. **2019**, 8(7):235. https://doi.org/10.3390/antiox8070235
15. Vandana, GD; Sejian, V; Lees, AM; Pragna, P; Silpa, MV; Maloney, SK. Heat stress and poultry production: impact and amelioration. *Int J Biometeorol*. **2021**, 65(2):163-179. doi:10.1007/s00484-020-02023-7
16. Li, Y; Wang, K; Li, C. Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. *Antioxidants*. **2024**, 13(11):1375. https://doi.org/10.3390/antiox13111375
17. Goel, A; Ncho, C-M; Jeong, C-M; Gupta, V; Jung, J-Y; Ha, S-Y; Yang J-K; Choi, Y-H. Alleviation of acute heat stress in broiler chickens by dietary supplementation of polyphenols from shredded, steam-exploded pine particles. *Microorganisms*. **2025**, 13(2):235. https://doi.org/10.3390/microorganisms13020235
18. Oni, AI; Adeleye, OO; Adebowale, TO; Oke ,OE. The role of phytogenic feed additives in stress mitigation in broiler chickens. *J Anim Physiol Anim Nutr (Berl)*. **2024**,108(1):81-98. doi:10.1111/jpn.13869
19. Ghazi, S; Amjadian, T; Norouzi, S Single and combined effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. *Int. J. Biometeorol.* **2015**, 59, 1019–1024. https://doi.org/10.1007/s00484-014-0915-4
20. Elbaz, AM; Ashmawy, ES; Ali, SAM; Mourad, DM; El-Samahy, HS; Badri, FB; Thabet, HA Effectiveness of probiotics and clove essential oils in improving growth performance, immuno-antioxidant status, ileum morphometric, and microbial community structure for heat-stressed broilers. *Sci. Rep.* **2023**, 13, 18846. https://doi.org/10.1038/s41598-023-45868-9
21. Kamboh, AA; Hang, SQ; Bakhetgul, M; Zhu, WY Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. *Poult. Sci.* **2013**, 92, 2411–2418. https://doi.org/10.3382/ps.2012-02960
22. Săracilă, M; Panaite, TD; Papuc, CP; Predescu, CN; Untea, A Dietary phytogenic mixture for broilers reared under thermoneutral and heat stress conditions. *Arch. Zootech.* **2020**, 23, 101–116. https://doi.org/10.2478/azibna-2020-0016
23. Attia, YA; Hassan, RA; Addeo, NF; Bovera, F; Alhotan, RA; Al-qurashi, AD; Al-Baadani, HH; Al-Banoby, MA; Khafaga, AF; Eisenreich, W; Shehata, AA; Basiouni, S Effects of Spirulina platensis and/or Allium sativum on antioxidant status, immune response, gut morphology, and intestinal lactobacilli and coliforms of heat-stressed broiler chicken. *Vet. Sci*. **2023**, 10, 678. https://doi.org/10.3390/vetsci10120678
24. Abou-Kassem, DE; Mahrose, KM; Meselhy, MA; Bassiony, SSM; Alhotan, RA; Attia, YA; Addeo, NF; Bovera, F; De Olivera, MC Assessment of green tea and/or ginger powder on growth performance, carcass traits, and blood profiles of broilers exposed to heat stress conditions for sustainable broiler farming. *Ann. Anim. Sci.* **2025**, 25, 213–224. https://doi.org/10.2478/aoas-2024-0071
25. Foucquier, J; Guedj, M Analysis of drug combinations: Current methodological landscape. *Pharmacol. Res. Perspect*. **2015**, 3, e00149. https://doi.org/10.1002/prp2.149
26. Bliss, CI The toxicity of poisons applied jointly. *Ann. Appl. Biol.* **1939**, 26, 585–615. https://doi.org/10.1111/j.1744-7348.1939.tb06990.x
27. Chou, TC Drug combination studies and their synergy quantification using the Chou–Talalay method. *Cancer Res.* **2010**, 70, 440–446. https://doi.org/10.1158/0008-5472.CAN-09-1947
28. Abdelli, N; Solà-Oriol, D; Pérez, JF Phytogenic feed additives in poultry: Achievements, prospective and challenges. *Animals* **2021**, 11, 3471. https://doi.org/10.3390/ani11123471
29. Pirmohamammadi, A; Daneshyar, M; Farhoomand, P; Aliakbarlu, J; Hamian, F Effects of Thymus vulgaris and Mentha pulegium on colour, nutrients and peroxidation of meat in heat-stressed broilers. *S. Afr. J. Anim. Sci.* **2016**, 46(3). https://doi.org/10.4314/sajas.v46i3.7
30. Fayed, RH; Ali, SE; Yassin, AM; Madian, K; Bawish, BM Terminalia bellirica and Andrographis paniculata dietary supplementation in mitigating heat stress-induced behavioral, metabolic and genetic alterations in broiler chickens. *BMC Vet. Res.* **2024**, 20, 388. https://doi.org/10.1186/s12917-024-04233-2

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

31. Mohammed, NI; Abdulkareem, ZA; Abdollahi, A; Khdir, HA; Ghaffar, OR; Ahmed, OR; Arif, RH; Mahmood, HA; Mustafa, SI; Khdhir, HB Effects of black cumin, fenugreek, and sesame seeds as a mixture on performance, intestinal morphology, and blood traits of broilers under chronic heat stress conditions. *Ital. J. Anim. Sci.* **2023**, 22, 1134–1150. https://doi.org/10.1080/1828051X.2023.2273426
32. Tekce, E; Çınar, K; Bayraktar, B; Takma, Ç; Gül, M Effects of an essential oil mixture added to drinking water for temperature-stressed broilers: Performance, meat quality, and thiobarbituric acid-reactive substances. *J. Appl. Poult. Res.* **2020**, 29, 77–84. https://doi.org/10.3382/japr/pfz030
33. Ruff, J; Tellez, G; Forga, AJ; Señas-Cuesta, R; Vuong, CN; Greene, ES; Hernandez-Velasco, X; Uribe, ÁJ; Martínez, BC; Angel-Isaza, JA; Dridi, S; Maynard, CJ; Owens, CM; Hargis, BM; Tellez-Isaias, G Evaluation of three formulations of essential oils in broiler chickens under cyclic heat stress. *Animals* **2021**, 11, 1084. https://doi.org/10.3390/ani11041084
34. Elbaz, AM Effect of linseed oil and fermented pomegranate peel supplementation on the growth performance, digestive function, and muscle fatty acid deposition of heat-stress broiler chickens. *J. Adv. Vet. Res.* **2023**, 13, 1881–1888.
35. Parandoosh, H; Khodaei-Motlagh, M; Ghasemi, HA; Khaltabadi Farahani, AH Effects of day-of-hatch intramuscular administration of a herbal extract mixture and its re-supplementation in drinking water on growth performance, stress indicators, and antioxidant status of broiler chickens reared under hot summer conditions. *Trop. Anim. Health Prod.* **2023**, 55, 196. https://doi.org/10.1007/s11250-023-03597-1
36. Shah, M; Chand, N; Khan, RU; Saeed, M; Ragni, M; Tarricone, S; Laudadio, V; Losacco, C; Tufarelli, V Mitigating heat stress in broiler chickens using dietary onion (Allium cepa) and ginger (Zingiber officinale) supplementation. *S. Afr. J. Anim. Sci.* **2022**, 52(6). https://doi.org/10.4314/sajas.v52i6.07
37. Madkour, M; Aboelenin, MM; Habashy, WS; Matter, IA; Shourrap, M; Hemida, MA; Elolimy, AA; Aboelazab, O Effects of oregano and/or rosemary extracts on growth performance, digestive enzyme activities, cecal bacteria, tight junction proteins, and antioxidants-related genes in heat-stressed broiler chickens. *Poult. Sci.* **2024**, 103, 103996. https://doi.org/10.1016/j.psj.2024.103996
38. Mohammed, FS; Uysal, İ; Sevindik, E; Doğan, M; Sevindik, M. Pimpinella species (Anise): Traditional use, Mineral, Nutrient and Chemical contents, Biological activities. *International Journal of Traditional and Complementary Medicine Research.* **2023**;4(2):97-105. doi:10.53811/ijtcmr.1306831
39. Bayraktar, B; Tekce, E; Aksakal, V; Gül, M; Takma, Ç; Bayraktar, S; Bayraktar, FG; Eser, G Effect of the addition of essential fatty acid mixture to the drinking water of the heat stress broilers on adipokine (Apelin, BDNF) response, histopathologic findings in liver and intestines, and some blood parameters. *Ital. J. Anim. Sci.* 2020, 19, 656–666. https://doi.org/10.1080/1828051X.2020.1778548

Article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)